APPENDIX 1 DATA SHEETS

FORM P – Primary stranding data

FORM C1 – Clinical data

FORM HI – Human interference

- FORM T1 Tier 1 sampling (live and deceased)
- FORM T2 Tier 2 sampling (deceased)
- FORM T3 Tier 3 sampling (for veterinarian or trained technician)

FORM T4 – Tier 4 (pathology)

MASS STRANDING :

- TEAM ONE DATA SHEET A
- TEAM ONE DATA SHEET B (PHOTOS of ADDITIONAL FEATURES)
- TEAM TWO DATA SHEET (MEASUREMENTS, SKIN, BLUBBER AND MUSCLE)
- TEAM THREE DATA SHEET- (TEETH, (See Separate Sheet) EYE AND EXTERNAL PARASITES)
- TEAM THREE- (TOOTH SAMPLES)
- TEAM FOUR DATA SHEET (2 PAGES plus PATHOLOGY)
- TEAM FOUR (PATHOLOGY) ONE FORM PER ANIMAL
- TEAM FOUR ALTERNATIVE (BULK RECORDING FORM) (one form, many animals)
- SUMMARY PRESERVATION, SHIPPING, TRACKING : For additional information see Appendix 4, and shipping info, tracking forms.

FORM SP - SHIPPING INFORMATION

SAMPLE PRESERVATION AND TRACKING FORM

Ρ

Necessary Equipment: Form, clipboard, pen, map/ GPS if possible, camera, phone, state operational procedures **Necessary personnel**: One person, low level skill required **Carcass code**: <u>All animals All species</u> **Additional information**: Guide/ form for human interference. Species ID guide, or call *see guide for definition of dependant young or contact a specialist (Appendix 2)

PRIMARY STRANDING DATA- describes	event- one form per event stranding ref #
collected by (name)	(contact)
LOCATION State Nearest Named	d Place
Distance+ direction	
Lat/ LongN	Map referenceDatum type
First seen:D/M/Y by (con	tact)at location
Reported:/ by	at location
Data collected / by	at location
STRANDING INFORMATION	
No of animals Total Male	Female Dependant young*
Species	Species confirmed?by
See species ID key at Appendix 4	
Time and date of stranding	estimate or definite? Seen alive? (Y/N)
Circumstances of stranding	
Weather at estimated/ definite time of actual st	tranding: Temp, cloud cover/ 8, Sunny?
wind speed wind dirnSea sta	ate, swell height dirn
Evidence of human interference: Boat collision	on Shot, Fisheries interaction Other
Puncture wound Cut Abrasion	Missing appendage Other
If evidence of human interaction, see protoc	col for human interference (HI).
No of animals rescued: Total Male	e Female Dependant young
Direction headed	
	by (contact)
Sampled Tier (1,2,3,4) Method of dispo	sal
Sketch of stranding site, including position of	f animals, indicate wind direction, swell direction and note time of skete
Take photo- Photo #s	
Photographer contact details	

If greater than 5 animals, and more than 6 people (some with experience) consider Mass Stranding sampling protocol (Appendix 3). If can be moved or time unlimited, and inexperienced veterinary expertise available, consider single dead sampling protocol (Appendix 2). Otherwise progress to tier 1. If time and equipment permit, tier 1,2. If time, equipment and personnel permit, tire 1,2,3. If experienced veterinary assistance available on site, tier 1,2,3,4. (see tier guide) If human interference (HI) do not proceed until HI protocol is completed and contact (insert state authority contact for compliance issues).

CI	tubes, syring Necessary p Carcass cod sampling. A	quipment: Monitoring: es, needles, bacto swabs ersonnel: Monitoring: (e: All animals All specie dditional information: nd particularly to mani	?, ice/ refrigeration, Dne person, preferab es. Additional infor Guide/ form for hun	plastic vials, plastic p ly experienced. Veter rmation: From SP, Ap han interference. Spec	ipettes, gloves. (NB p rinarian required for s ppendix 1. coordinat sies ID guide, or Appo	ermits for some sam ampling. a with rescue and t endix 2. NB: ethics	pples) ier one data and permit is required
CLINICAI	DATA	stranding ref#_	c	ollected by			
Animal ID_		_ Sex	Adult/ Juv	Seen al	ive?Died	:	
Time/ date of	of this recor	d:D	/M/Y, o	contact details			
Species			confirm	ed by			
* every 10-	15 min. If f	easible, collect bloc	d at least twice	(beware safety).	<u>Underlined</u> = price	ority	
Parameter		Conditions	Response	Time of assessment	Time of assessment	Time of assessment	Time of assessment
				Response	Response	Response	Response
Muscle tone		Attempting to open mouth; ability to move	resistance to traction of the tongue;				
Respiration rate/ m	in	1-3 / min					
Respiration quality		< 1 second					
<u>Vocalizations</u>		Audible (apart from respirations)					
Heart rate/min		30 -70bpm					
Auscultate or palpa posterior to pectora level of carpus:							
Eye reflex		Should have					
Palpebral		reflex lid closure to touch. Note any discharges					
Blowhole reflex		Should close if					
Should be closed		touched					
Anal tone		Should tighten when touched					

Temperature

– not glass) **SAMPLES** <u>Blood LiH</u> Blood EDTA Blood Fl Ox Blood plain

Only useful is suitable rectal

probes available (min 30 cm

Skin scraping (DNA)

Biopsies (edge of lesion)

Parasites

Swabs (chill) Blowhole spray Normal 36.5 – 37.5C (97.7 –

(ethics permits required)

(ethics permits required)

(check orifices and grooves - label and note where found)

(On sterile agar plate /swab) Time collected......

Blowhole...... Discharges...... Lesions...... Time collected.....

99.5F)

External observations (skin, eyes, orifices, body condition)	Time of: Death	Release	
A dorsa 1	B tail stock	pec.	tail

Monitoring details: Codes for reflexes/ tone/ resp- S = strong, W = weak, N = none.

Sample details: <u>Skin</u> (DNA)- scrape plastic scourer pad along animal to collect skin slough. Preserve in 80% ethanol or freeze. Avoid freeze-thaw.

<u>Blood</u>: 2 ml LiH to lab ASAP <48h chilled. If collected, send EDTA blood and 2 ml each separated LiH plasma and serum (separate by centrifuge 3000rpm 10 min, or stand to settle). Freeze remaining serum, plasma, blood cells. If lab > 48h, send frozen plasma/ serum and fresh whole blood smear.

Swabs: Send chilled to lab ASAP < 48h. If longer, freeze. Request CBC, full biochem. Biopsy: 10x vol 10% formalin. Additional information: Species ID guide, or contact a specialist (Appendix 4).

	-

To be followed for all animals where human interference is a possibility, either before or after death. Coordinate this protocol with tier 1. Do not proceed to T2-T4 until HI is satisfied. It does not replace existing state operational procedures for compliance issues, but is intended as a link between the sampling protocols and those procedures.

HUMAN INTERFERENCE

Stranding ref#	Carcass c	ode			
Animal ID	Sex		Seen alive?	Died:	
Time/ date of this record	:	//,	collected by (name)	(contact)	
Species		confi	rmed by		
1. Compliance incident is BoatShot	· · · · · · · · · · · · · · · · · · ·	, possibleOther	_ or unlikely		

2. Contact (insert compliance officer/ coordinator contact) to seek advice before retrieval or necropsy.

3. Ensure at least one member of Conservation agency, Parks or Fisheries staff present at all times.

4. Treat the animal, surrounding area and any samples from the animal as **potential evidence** and ensure staff in the field use evidence collecting guidelines.

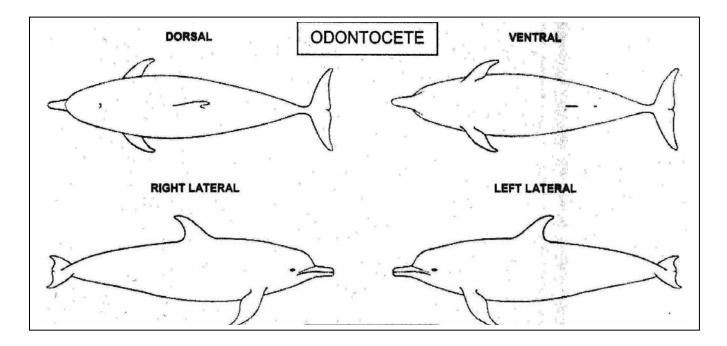
5. Without disturbing the animal, complete as much of forms P (Primary stranding data) and T1 individual ID, photos etc) as possible (especially all photographs, with additional photographs of any surrounding evidence). All photos to include label with animal ID and date.

6. **Document with notes and photographs**, any manipulations of the animal ie, movement, sampling, post-mortem examination.

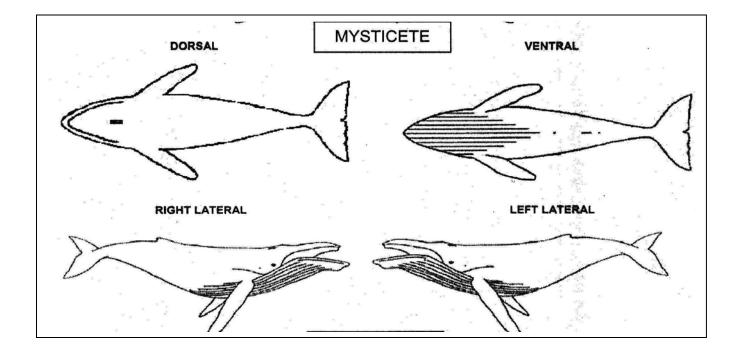
8. Ensure **as detailed a necropsy as possible,** including pathology. Ensure at least one member of **Parks staff** present at all times. Contact person from Appendix 2 for **specialist necropsy**. Only if not available, follow protocols appropriate to resources (after photography and description, parts of the animal may be transported to a pathologist for examination of suspicious lesions- use sample tracking forms). Chilled < 48h, frozen if >48h to pathologist) N/E = not examined

Initial observation	Yes	No	unsure	N/E	Photo ##
Body condition: robust					
emaciated					
Penetrating wounds					
Cuts					
Abrasions					
Missing appendages					
Haemorrhage/ bruising					
Scavenger damage					
Sub-dermal haemorrhage					
Broken bones					
Lung contents: fluid					
Froth					
Stomach contents unusual					
Other					

Description/ sketches/ other details:



Diagrams: Victorian Cetacean Contingency Plan (Warneke, Obendorf and Gallechio)



vial, Salt soln/ 80% ethanol/ freezer. Necessary personnel: One- two per Carcass code: All animals. Coordinate with rescue, welfare and clinic Additional information: Guide/ form (HI) for human interference; Appendic contact a specialist (Appendix 4), guide to dependent young. If live: clinic (P) is complete.	ical sampling dix 2 (single animal protocol). Species ID guide, or
TIER ONE SAMPLING (live* and deceased) stranding ref #	Collected by
Animal ID Sex Adult/ Juv Sec	
Time/ date of this record//, contacts	
Species Carcass code (see foo	ter below) Underlined- priority phote
EVIDENCE OF HUMAN INTERFERENCE : Boat collision Shot	t, Fisheries Other
Puncture wound Cut Abrasion Missing appendage	e Other
Evidence of human interaction? If so, see protocol for human interfer	
Photos (right angle to animal, preferably digital, colour, avoid flash, i	include animal ID and scale in photo)
1. WHOLE ANIMAL SIDE VIEW left # right #	- <u>-</u>
2. Tail flukes (above) #	Female Imammary a umbilio
3. Dorsal fin # 4. <u>HEAD FROM SIDE</u> left #right #	the source of th
5. Head from above $\#$	
6. <u>Teeth/ baleen</u> #	genita genita genita bationy bationy
7.Whole ventral surface#8.Genital slit, anus and umbilicus (all in one)#	
8. Genital slit, anus and umbilicus (all in one) # 9. Scars, wounds, injuries, other abnormalities #	
10. Flipper left #right #	
11. External parasites (check grooves and orifices) $\#$	•
Measurements: (straight line) in mm	
1.TOTAL LENGTH (tip of upper jaw to deepest part of fluke notch)	
2. Tip upper jaw- centre of eye	
3.Length of gape (upper jaw to corner mouth)	
4. Tip upper jaw to blowhole	
5. Tip upper jaw to front insertion of flipper	7/=)
6. Tip upper jaw to tip dorsal fin	
7. Tip upper jaw to centre anus	
8.Max girth	
9.Flipper- tip to front insertion	
10.Flipper- max width	
11.Tail flukes tip to tip	
12. Depth of fluke notch	27
13.Dorsal fin tip to base	24
Throat grooves? feathering of tongue?Snout hairs?	(Y/N)
Weight (code 1*, 2 or 3)	
Count teeth/baleen from front to back and enter any missing teeth in seq	guence i.e. 10 (2) 9 (4) is 10 teeth followed by
missing, followed by 9 present, etc. Note if teeth not erupted.	Discharges (describe):
	Eye
UR UL	BIOWINIE
LR LL	
Milk present/ absent (cut mammary gland if dead)	Genital
SAMPLE:SKIN SCRAPING/ SLICE FOR DNA X 2*(*if alive, do not collect unless ethics permit has been obtained)	Other
Sample details: <u>Skin</u> (DNA)- live-scrape plastic scourer pad along animal to colle	Let align alough or dood collect 2mm doon "aboose

Carcass code: 1, alive. **2**, fresh dead. **3**, Some bloating may be present, possibly with tongue or penis protruded; mild odour, mucous membranes dry; eyes shrunken. **4**, decomposing, organs disintegrating, sloughing of skin; strong odour, blubber soft, pockets of gas or oil; muscle easily torn.

Т2	Necessary Equipment: Form, pen, camera, scale and ID, large/ flensing knives, meat hooks, ruler/ callipers, plastic bags, ice/ refrigeration. Preservation: 100% ethanol for cleaning, Teflon sheeting/ bags or glass jars. Zip-lock bags, aluminium foil, freezer. Cutting board, stainless/ titanium blades. labelling pen, dymo labels or pencil on card for internal labels. Necessary personnel: One- two people, moderate skill required Carcass code: Code 2-3, some to 4 (see below). Additional information: Guide/ form for human interference; Appendix 2, Species ID guide, or contact a specialist (Appendix 4). For sample preservation: summary form SP or Appendix 4.						
TIER T	WO SAMPLING (deceased)	stranding ref #	collecte	ed by			
Animal 1	D Sex	Adult/ Juv	Seen alive?	Died:			
Time/ da	te of this recordD_	/M/Y, contact	details		_		
Species_		confirmed by					
(for spec	ies ID key see Appendix 4)						

Carcass code

A

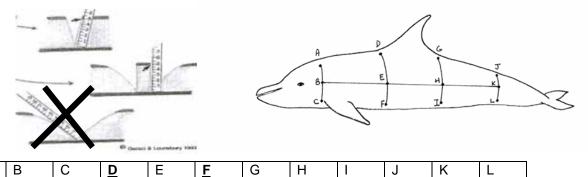
<u>20cm x 20cm block of skin and full thickness blubber (codes 2-3)</u> label with ID and hold chilled. □
 (Anterior to dorsal fin. Sample to be trimmed later. If oil leaching out of sample then blubber probably useless.)

 <u>6cm x 6cm x 6 cm block of muscle (codes 2-3)</u> from under site of blubber sample, label and hold chilled □
 3. <u>1 cm thick slice of external lesions (include edge) in 10x vol 10% buffered formalin for histopathology</u>

 Describe lesion: Photo# Label sample □

4. <u>External parasites (codes 2-4)</u> Check all orifices and grooves for parasites Collect in 70% ethanol or 10% formalin. Label with animals ID and the location that the parasite was found.

5. Blubber measurements (codes 2-4): refer to diagram. Measurements to be stated in millimetres to the nearest 0.1mm. Measure from the base of the skin to the surface of the muscle. Ensure measurement is at right angles to underlying muscle and skin surface. Priority measurements are D and E.



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incise ti	nrougn ti	re biubbe	er to the i	nuscie ad	coraing	to the au	igram ab	ove ana i	neasure	diudder i	nickness	(in mm) at
							-				<u> </u>	

Incise through the blubber to the muscle according to the diagram above and measure **blubber thickness** (in mm) at the specified locations. Ensure thicknesses mid-dorsal just in front of dorsal fin (D) and mid-ventral just in front of umbilicus (F) are measured as a minimum. Collect **parasites** and label with ID and where found. \Box

Remove head or Jaw (head easiest unless really big)

Contact specialist (Appendix 2) for decision on brain/ ear removal,

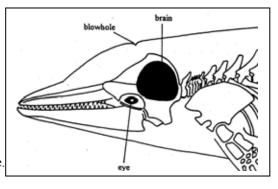
radiography, or preservation.

Don't use chainsaw (dangerous and damaging). Note anatomy.

Cut behind skull, down to spine, then disarticulate

(some skill required here, cut between vertebrae).

Pack in ice/ refrigerate. If only taking jaw: place in plastic bag, freeze.



Sample details: FOLLOW EXACTLY Remove skin/ blubber/ muscle to clean environment (KEEP CHILLED). <u>Surface skin</u> (dark layer-305mm deep) 15mmx15mm diced finely in saturated salt +/-10% DMSO, or 80% ethanol, or freeze x2; 3cm x 1cm in vial and freeze x 3. <u>Blubber</u>: Rinse Teflon sheeting/ glass jars/ blades/ cutting surface with 100% ethanol between tissues. Trim off contaminated outer surface. 100g in glass or wrap in Teflon/ alfoil then in plastic zip lock x 5, 100g in plastic (no alfoil), 1cmx1cmx full thickness in plastic x2 (all samples full thickness, include deep skin to ID outer layers). Remove as much air as possible (label inside and out). Freeze -20C (-80 if possible). <u>Muscle</u>: 5mm cube in 10x vol sat salt soln +/- 10%DMSO or 80% ethanol or freeze; freeze 1cmx 1cm x 3 cm in plastic. NO internal labels to contact specimens (double bag and put label between) <u>Parasites</u>- relax in fresh water then ethanol **More information** Species ID guide, or contact a specialist (Appendix 4), sample shipping, tracking forms.

 Necessary Equipment: Form, pen, camera (prefer digital), large/ flensing knives, meat hooks, ruler/ callipers, plastic bags, ice/ refrigeration. Preservation: 80-100% ethanol for cleaning, Teflon sheeting/ bags or glass jars. Zip-lock bags, aluminium foil, freezer. Cutting board, stainless/ titanium blades. 10% formalin. 100% ethanol for stomach contents (or freeze if don't want algae). Accurate weighing scales. labelling pen, dymo labels or pencil on card for internal labels Necessary personnel: > two people, dissection safety skill required, including understanding of zoonoses. Carcass code: Code 2-3, some to 4 (repro tract, stomach contents). Additional information: Appendix 2 (single animal protocol)
TIER THREE SAMPLING (if veterinarian or trained technician present, do concurrent with tier 4)
Stranding ref# Carcass code(note step 1 for code 2-3 only- remainder 2-4)
Animal ID Sex Adult/ Juv Seen alive? Died:
Time/ date of this record D/M/Y, collected by (name) (contact)
Species confirmed by
 1.Chilled organs: (CODE 2-3 ONLY). Label with ID and organ and chill- to be trimmed later. Liver (left hind) 500g, Kidney (left hind) 500g, Spleen 500g, Heart blood 20-50 ml 2. Reproductive organs: (CODE 2-4) Remove entire reproductive tract and mammary glands for processing later (label and chill) OR if too large:
collect gonads - weigh and measure both (Testis- weigh and measure testis and epididymis separately; Examine ovaries for CL/ follicles, weigh and measure, label and keep chilled for preservation later)
Rt Gonad: Weight,x, Features
L Gonad: Weight,x, Features
Rt Uterine horn: Digmeter Woll thickness length

Label and store in 10x vol 10% formalin. If testis or uterus are very large, take a cross- and longitudinal section of the testis and representative portions (1cm thick) of the uterus (vagina, cervix, uterine horn, fallopian tube)

length

Fetus samples: Skin_	Blubber	_Muscle	Other
Fetus measurements:	(if small enough freeze	e whole after	r measuring and labelling)
Total length	Nose	e to anus	
Flipper length	Fluke tip-ti	р	Max Girth

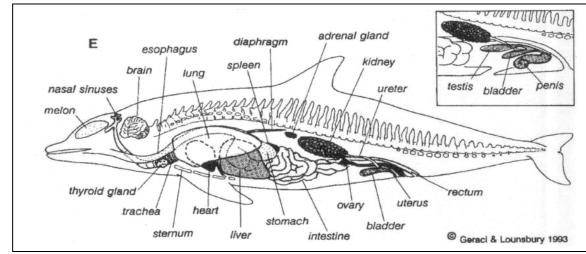
Diameter Wall thickness

3. Stomach contents first stomach _____ second ____ third _____ 100g freeze. 100g in ethanol (3 parts digesta, 1 part 100% ethanol or 2 part digesta, 1 part 80% ethanol). Bag and freeze remainder (in stomach) or if too large, weigh all and store some as described, and sieve remainder. weight of contents first ______ second ______ third _____

4. Faeces (code 2 only) label and freeze some.

L Uterine horn:

5. Internal parasites. Relax in fresh water, then preserve in 80% ethanol or 10% formalin. Label with ID and where found______



Sample details: Remove samples to clean environment .For toxins: Rinse Teflon sheeting/ glass jars/ blades/ cutting surface with 100% ethanol between tissues. Trim off contaminated outer surface. <u>100g in glass or wrap in Teflon/ alfoil then in plastic zip lock-</u> Liver (organics). <u>20g in plastic (no metal, note blade metal)-</u> Liver, Kidney, Spleen (heavy metals). <u>100g in plastic-</u> Liver, Kidney (biotoxins). <u>1cmx1cmx3cm in plastic-</u> Liver Kidney, Spleen (stable isotopes). Remove as much air as possible (label inside and out). Freeze -20C (-80 if possible). Freeze remainder of Liver, Kidney, Spleen in plastic (virology). NO internal labels to contact specimens (double bag and put label between). Blood- centrifuge 3000rpm 10 min, freeze serum (liquid). Repro tract samples in 10% formalin (10x vol, no thicker than 15mm, slice larger tissues). **More information** Species ID guide, or contact a specialist (Appendix 4).

T4

Necessary Equipment: Form, pen, camera (prefer digital), large/ flensing knives, meat hooks, ruler/ callipers, plastic bags, ice/ refrigeration. Bacto swabs, blood tubes, sample pots, ziplock bags, forceps, scissors, scalpels, labelling pen, dymo labels or pencil on card for internal labels. Microscope slides. Preservation: 10% formalin. 100% ethanol for stomach contents. **Necessary personnel**: > two people. Pathologist, vet or zoologist experienced in cetacean dissection (including understanding of zoonoses).

Carcass code: Code 2-3, some to 4 (gross pathology only). Additional information: from SP, Appendix 2

TIER FOUR (PATHOLOGY)- pathologist, vet or zoologist experienced in cetacean dissection (including understanding of zoonoses). Tiers 1,2,3 must be completed

External	Description	Labelled (tick)	Photo #	Fluid	Tissue	Swab
Eye						
Blowhole						
Mouth						
Anus						
External lesions						
Other						

Superficial	Description	Labelled (tick)	Photo #	Fluid	Tissue	Swab
Mammary						
Subcutis						
Muscle						
AxillaryLN						
Brachial nerve						

Open	Description	Labelled (tick)	Photo #	Fluid	Tissue	Swab
Pleural cavity						
Abdominal cavity						

Thorax	Description	Labelled (tick)	Photo #	Fluid	Tissue	Swab
Pericardium						
Heart/ blood						
Thyroid						
Oesophagus						
Trachea						
Lung						
Thoracic LN						
Thymus						

Abdomen	Description	Labelled (tick)	Photo #	Fluid	Tissue	Swab
Liver						
Spleen						
Pancreas						
Mesenteric LN						
Kidneys						
Adrenals						
Bladder						
Reproductive tract	See tier three					
Gastro-intestinal						

Additional description of lesions:

Recommend: Collect all tissues and edges of lesions and chill for placement in 10x vol 10% formalin off beach (not thicker than 15mm). Additionally, excise lesions, kidney lung and spleen as 6cmx6cmx6cm pieces and place in sterile container and chill, then, off beach, sear surface, cut through with sterile swab, and swab inside cut. Swab to lab chilled <72hours. Request aerobic and anaerobic culture. Make impression smears with cut surfaces. Cut remainder in half- freeze half, other half in formalin.

IDSEXLactPreg Y/NPhoto#: TailPhoto#: EntirePhoto#: genitalsCarcass Code/ timeIII <t< th=""><th>ID</th><th></th><th></th><th>Drag</th><th></th><th colspan="5">Photos from perpendicular to subject.</th></t<>	ID			Drag		Photos from perpendicular to subject.				
	ID	SEX	Lact	Preg	Photo#:	Photo#:	Photo#:			
			Y/N	Y/N	Tail	Entire	genitals	Code/		
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DATA SHEET A, TEAM ONE (MASS STRANDING)

Record both film#/ frame #. Take photos from perpendicular to subject.

DATE____LOCATION_____ TOPOGRAPHY PHOTOS#_____ COMMENTS_____

DATA SHEET B,TEAM ONE- MASS STRANDING (PHOTOS of ADDITIONAL FEATURES) DATE____LOCATION____

ID	FILM	FRAME	DESCRIPTION

DATA SHEET TEAM TWO- MASS STRANDING MEASUREMENTS, SKIN, BLUBBER AND MUSCLE

DATE LOCATION

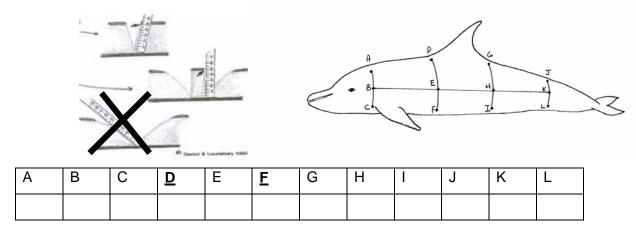
Id T1 Flippe Df Flukes Girth Blub Muscle Blubber La Skin Thickness Time Time Time r

(1) **TL**- Total length, tip of upper jaw to the deepest part of the notch between the tail flukes.

- (2) LA- Length, tip of upper jaw to the centre of the anus
- (3) **FLIPPER**-Length of flipper, anterior insertion to tip
- (4) **DF**-Height of dorsal fin, tip to base
- (5) **FLUKE**-Span of tail flukes, tip to tip. If whole span cannot be taken, take half-span (from fluke notch to tip) and state. Do not take a half span and simply double it!
- (6) **GIRTH**-Girth, maximum. If whole girth cannot be taken, take half girth and state. If animal has been on beach for some time and has started to bloat, **do not take this measurement**, state in notebook that animal is bloated.

1. Blubber measurements (codes 2-4): refer to diagram. Measurements to be stated in millimetres to the nearest

0.1mm. Measure from the base of the skin to the surface of the muscle. Ensure measurement is at right angles to underlying muscle and skin surface.



Incise through the blubber to the muscle according to the diagram above and measure **blubber thickness** (in mm) at the specified locations. Ensure thicknesses mid-dorsal just in front of dorsal fin (D) and mid-ventral just in front of umbilicus (F) are measured as a Minimum.

- If time is of the essence make sure that at least a total length is taken (measurement #1).
- ALL MEASUREMENTS TO BE TAKEN IN STRAIGHT LINE- DO NOT FOLLOW CURVE.

TEAM THREE DATA SHEET- MASS STRANDING TEETH, (See Separate Sheet) EYE AND EXTERNAL PARASITES

DATE____LOCATION_____

ID	D	East and a 1 D.	Manulau ef T
ID	Eye	External Parasites	Number of Teeth
	time	(note where found)	samples (see attached
			sheet)
<u> </u>			
ļ			
<u> </u>			

TEAM THREE- MASS STRANDING TOOTH SAMPLES DATE_____LOCATION______

ID	Upper R	Upper L	Lower R	Lower L
0 1 1				
Sampled tooth#- tooth#				
100111#- 100111#				
Sampled				
tooth#- tooth#				
Sampled				
tooth#- tooth#				
Sampled				
tooth#- tooth#				
Sampled				
tooth#- tooth#				
Sampled				
tooth#- tooth#				
Sampled				
tooth#- tooth#				
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tooth#- tooth#				
Sampled				
tooth#- tooth#				
Sampled				
tooth#- tooth#				

(Sampled tooth#-tooth#= mark which teeth sampled from- to)

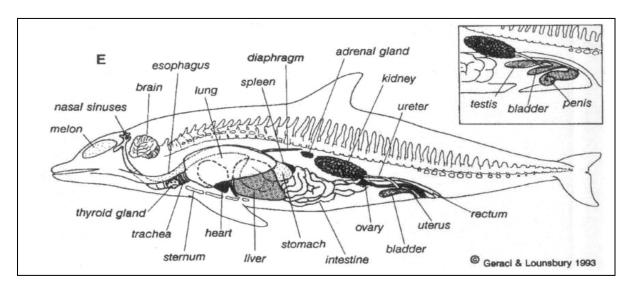
TEAM FOUR DATA SHEET MASS STRANDING (2 PAGES plus PATHOLOGY)

One per animal

Necessary Equipment: Form, pen, camera (prefer digital), large/ flensing knives, meat hooks, ruler/ callipers, plastic bags, ice/ refrigeration. Preservation: 100% ethanol for cleaning, Teflon sheeting/ bags or glass jars. Zip-lock bags, aluminium foil, freezer. Cutting board, stainless/ titanium blades. 10% formalin. 100% ethanol for stomach contents (or freeze if don't want algae). Accurate weighing scales. labelling pen, dymo labels or pencil on card for internal labels Necessary personnel: > two people, dissection, safety skill required, including understanding of zoonoses. Carcass code: Code 2-3, some to 4 (repro tract, stomach contents).Additional information: Appendix 2, form SP
Stranding ref# Carcass code
(note step 1 for code 2-3 only- remainder 2-4)
Animal ID Sex Adult/ Juv Seen alive? Died:
Time/ date of this recordD/M/Y,
collected by (name)(contact)
Species confirmed by
1. Chilled organs: (CODE 2-3 ONLY). Label with ID and organ and chill- to be trimmed later.
Liver (left hind) 500g, Kidney (left hind) 500g, Spleen 500g,
Heart blood 20-50 ml
2. Fetus samples: SkinBlubberMuscleOther
Fetus measurements: (if small enough freeze whole after measuring and labelling)
Total length Nose to anus
Flipper length Fluke tip-tip Max Girth
3. Reproductive organs: (CODE 2-4) Remove entire reproductive tract and mammary glands for processing later (label and chill) OR if too large: collect gonads - weigh and measure both (Testis- weigh and measure testis and epididymis separately; Examine ovaries for CL/ follicles, weigh and measure, label and keep chilled for preservation later)
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Remove entire reproductive tract and mammary glands for processing later (label and chill) OR if too large: collect gonads - weigh and measure both (Testis- weigh and measure testis and epididymis separately; Examine ovaries for CL/ follicles, weigh and measure, label and keep chilled for preservation later)
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5. Faeces (code 2 only) label and freeze some.

6. Internal parasites. Relax in fresh water, then preserve in 80% ethanol or 10% formalin. Label with ID and where found



Sample details: Remove samples to clean environment .For toxins: Rinse Teflon sheeting/ glass jars/ blades/ cutting surface with 100% ethanol between tissues. Trim off contaminated outer surface. <u>100g in glass or wrap in Teflon/ alfoil then in plastic zip lock-</u> Liver (organics). <u>20g in plastic (no metal, note blade type)-</u> Liver, Kidney, Spleen (heavy metals).. <u>100g in plastic-</u> Liver, Kidney (biotoxins). <u>1cmx1cmx3cm in plastic-</u> Liver Kidney, Spleen (stable isotopes). Remove as much air as possible (label inside and out). Freeze -20C (-80 if possible). Freeze remainder of Liver, Kidney, Spleen in plastic (virology). NO internal labels to contact specimens (double bag and put label between). Blood- centrifuge 3000rpm 10 min, freeze serum (liquid). Repro tract samples in 10% formalin (10x vol, no thicker than 15mm, slice larger tissues).

TEAM FOUR (PATHOLOGY)- pathologist, vet or zoologist experienced in cetacean dissection (including understanding of zoonoses). (ONE FORM PER ANIMAL)

Necessary Equipment: Form, pen, camera (prefer digital), large/ flensing knives, meat hooks, ruler/ callipers, plastic bags, ice/ refrigeration. Bacto swabs, blood tubes, sample pots, ziplock bags, forceps, scissors, scalpels, labelling pen, dymo labels or pencil on card for internal labels.

Preservation: 10% formalin. 100% ethanol for stomach contents.

Necessary personnel: > two people. Pathologist, vet or zoologist experienced in cetacean dissection (including understanding of zoonoses).

External	Description	Labelled (tick)	Photo #	Fluid	Tissue	Swab
Eye						
Blowhole						
Mouth						
Anus						
External lesions						
Other						

Superficial	Description	Labelled (tick)	Photo #	Fluid	Tissue	Swab
Mammary						
Subcutis						
Muscle						
AxillaryLN						
Brachial nerve						

Open	Description	Labelled (tick)	Photo #	Fluid	Tissue	Swab
Pleural cavity						
Abdominal cavity						

Thorax	Description	Labelled (tick)	Photo #	Fluid	Tissue	Swab
Pericardium						
Heart/ blood						
Thyroid						
Oesophagus						
Trachea						
Lung						
Thoracic LN						
Thymus						

Abdomen	Description	Labelled (tick)	Photo #	Fluid	Tissue	Swab
Liver						
Spleen						
Pancreas						
Mesenteric LN						
Kidneys						
Adrenals						
Bladder						
Reproductive tract	See tier three					
Gastro-intestinal						

Additional description of lesions:

Recommend: Collect all tissues and edges of lesions and chill for placement in 10x vol 10% formalin off beach (not thicker than 15mm). Additionally, excise lesions, kidney lung and spleen as 6cmx6cmx6cm pieces and place in sterile container and chill, then, off beach, sear surface, cut through with sterile swab, and swab inside cut. Swab to lab chilled <72hours. Request aerobic and anaerobic culture. Make impression smears with cut surfaces. Cut remainder in half- freeze half, other half in formalin.

ALTERNATIVE (BULK RECORDING FORM) FOR TEAM FOUR- SEE OTHER FORMS AND PROTOCOL FOR INSTRUCTIONS. (one form, many animals)

Ð	Blubber dorsal (mm)	Blubber ventral (mm)	Liver 500g	Kidney 500g	Spleen 500g	Blood 20ml	Gonad L/R (g)	L Uterus diam/ wall/length (mm)	R Uterus diam/ wall/length (mm)	Stomach contents (wt, 1/2/3)	Contents collected?	Faeces	Parasites

SUMMARY PRESERVATION, SHIPPING, TRACKING : additional information see Appendix 4, and shipping info. tracking forms.

info, tracking forms.			-					
Sample/ Use	Quantity	Storage method	Carcass code					
			1	2	3	4	5	
SKELETAL, TEETH, BALEEN,								
FLIPPER								
Speciation, age HEAD (acoustic trauma)		Dried or frozen See protocol and species list		+	+	+	+	
Eye lens (age)		frozen		+				
Eye lens (age) Earplugs (age)		10% formalin		+	++			
SKIN		1078 10111141111		+	+			
Genetics (speciation, population	15mmx 15mm	$10x$ vol saturated salt soln \pm	+	+	+	+	+	
structure)	Tommx Tomm	10%DMSO. (or frozen or 80%	т	т	Ŧ	т	т	
structure)		ethanol)						
Stable isotopes (diet)	3cmx 3cm	frozen		+	+	+	+	
BLUBBER						·		
Organic contaminants-	100g (400g for archive)	Trimmed, frozen in glass/ teflon		+	+			
Biotoxins-	100g	Trimmed, frozen in plastic		+				
Fatty acids (diet)	1cmx 1cmx full thickness	Frozen		+	+			
Hormones	1cmx 1cmx full thickness	Frozen		+	+			
MUSCLE				-				
Genetics	15mmx 15mm	10x vol saturated salt soln ±		+				
		10%DMSO. (or frozen or 80%						
		ethanol)						
Stable isotopes (diet)	1cm x 1cm x 3cm	Frozen		+	+			
LIVER								
Organic contaminants-	100g	Trimmed, frozen in glass/ teflon		+	+			
Inorganic contaminants (metals)-	20g	Trimmed, frozen in plastic		+	+			
Biotoxins-	100g	Trimmed, frozen in plastic		+				
Polyaromatic hydrocarbon	5 mL bile, 50g liver- early	Liquid N, protect from light		+				
contaminants	class 2 only							
Virology	6 cm^3	Trimmed and frozen		+				
Stable isotopes (diet)	1cm x 1cm x 3 cm	frozen		+	+			
KIDNEY					† ·			
Inorganic contaminants (metals)-	20g	Trimmed, frozen in plastic		+	+			
Biotoxins-	100g	Trimmed, frozen in plastic		+	· ·			
Virology	6 cm ³	Trimmed and frozen		+				
Stable isotopes (diet)	1cm x 1cm x 3 cm	frozen		+	+			
SPLEEN		nozen		· ·	<u> </u>			
Virology	6 cm ³	Trimmed and frozen		+				
Bacteriology	6 cm ³	Chilled or seared and swabbed		+				
Stable isotopes (diet)	1 cm x 1 cm x 3 cm	frozen		+	+			
BRAIN		nozen			<u> </u>			
Organic contaminants-	100g	Trimmed, frozen in glass/ teflon		+	+			
Biotoxins-	100g	Trimmed, frozen in glass, tenon Trimmed, frozen in plastic		+	Т			
Virology	6 cm^3	Trimmed and frozen		+				
CHILLED ABNORMAL TISSUE	0 cm			т				
	6 cm ³	Chilled or seared and swabbed		-				
Virology	6 cm ³	Trimmed and frozen		++				
Virology (electron microscopy)	1mm ³	Chilled, glutaraldehyde 5%		+				
FORMALIN FIXED TISSUES		Chinea, gradialachyac 570	+	+			-	
Histopathology, histoanatomy		Room temp, formalin. Label inside	+	+	+		-	
mstopathology, instoanatolliy		and out	+	+	+		1	
STOMACH CONTENETS		All frozen or	+	<u> </u>		-	\vdash	
Otoliths		Frozen/ ethanol	+	+	+	+	\vdash	
Squid beaks		formalin	+	++	+	+ +	-	
Biotoxins			+	++	+	<u> </u>	-	
BLOOD			-	-	1		-	
EDTA/ LiH (Haematology)	> 1 mL	Chilled to lab in 48h	+	<u> </u>		<u> </u>	\vdash	
Plain. LiH and Fl Ox Plasma/ serum	> 1 mL > 5mL	Frozen	+ +	+	+		1	
(Biochemistry, serology, hormones)	- 51112	1102011	T	T	- T		1	
Toxicology	6 ml whole blood	Frozen	+	+	1	1	1	
SWABS (bacteriology)		Cool, to lab in 72h	+	++		<u> </u>	-	
PARASITES		Cooi, w 100 ill /211	- T	- T		<u> </u>	-	
Most		AFA and 80% ethanol	+	+	+		1	
Trematodes and cestodes		Fresh water then 10% formal saline	-				1	
FAECES (parasites, hormone) (Code	5g each	Frozen and 10% formalin	+ +	++	++		-	
2:DNA-freeze)	Jg cach		+	+	+		1	
			-	<u> </u>	+ .	-	\vdash	
		Measure weigh 10% tormalin (anon						
REPRODUCTIVE		Measure, weigh, 10% formalin (open uterus and section testis (not ovaries)		+	+			

SHIPPING INFORMATION

Additional information is contained within the IATA regulations for shipping dangerous goods. The following is a guide only. You should check with the relevant shipping/ customs/ environmental/ quarantine agents or sample recipients for the relevant permitting/ packaging and storage requirements of your state or agent. (NB: IATA approved shipping containers can be purchased)

- Make arrangements for samples to be maintained at storage temperature during shipping. Especially avoid freeze/ thaw.
- Ensure the recipient will be there to receive the samples
- For samples in liquid preservative (eg formaldehyde), preserve for approx 1 week then remove the liquid and wrap sample in gauze swab or cloth moistened with the preservative and seal container well.
- Double-bag samples with a label between (paper tag, pencil) and outside (lab marker) the bags.
- Place in a robust container (with dry ice if appropriate).
- Place in Styrofoam packed box or esky with appropriate coolant.
- In general, sample must be within 2 sealed, impact resistant containers with sufficient absorbent material to absorb all liquid enclosed in case of leakage.
- Enclose copies of relevant data forms. (keep a copy)
- Complete tracking form and enclose a copy (keep a copy)
- Ensure copies of relevant permits are attached on the outside of the package for inspection by authorities

64 Limited classes of dangerous goods which may be carried (Australia Post)

64.1 Dangerous goods specified in clauses 64.2 to 64.3 inclusive, may be lodged and carried by post providing they are properly packed and comply with such terms and conditions governing their carriage.

64.2 **Infectious perishable biological substance** which are dangerous goods may be lodged and carried by post within Australia providing:

64.2.1 an article containing such a substance is:

- (i) addressed to a recognised laboratory, hospital, clinic or regulatory body;
- (ii) lodged at an office for delivery within Australia by:-
 - 1 a qualified medical practitioner or veterinary surgeon;
 - 2 hospital, clinic, regulatory body or recognised laboratory;
 - 3 a member of a Commonwealth, State or Territory police force; or
 - 4 a person who has the authority of the agencies in clause (ii) above;
- (iii) sent at the perishable infectious biological substances rates determined by Australia Post; and
- (iv) packaged and presented in the manner prescribed in the current Technical Instructions of the Civil

Aviation Organisation as reflected in the IATA Dangerous Goods Regulations;

64.3 Non-infectious perishable biological substances and solid carbon dioxide (dry ice), when used as a refrigerant, may be lodged and carried by post within Australia providing:

64.3.1 an article containing such a substance is:

(i) addressed to a recognised laboratory, hospital, clinic or regulatory body;

- (ii) lodged at an office by:-
 - 1 a qualified medical practitioner or veterinary surgeon;
 - 2 hospital, clinic, regulatory body or recognised laboratory;
 - 3 a member of a Commonwealth, State or Territory police force; or
 - 4 a person who has the authority of the agencies in clause (ii) above; and

(iii) packaged and presented in the manner prescribed in the current Technical Instructions of the Civil Aviation Organisation as reflected in the IATA Dangerous Goods Regulations.

SAMPLE PRESERVATION AND TRACKING FORM Stranding ref #_____

 Date_____
 Location of stranding _____

Location of preservation_____
 Processed by _____

Method of storage before processing_____

Animal ID	Sample #	Carcass code	Tissue	Time/ Date processed	Method preservation	Sent to	Sent by (person/ date)	Arrived (person/ date)

NB: toxicology samples note blade metal, type of alcohol used (reagent grade or domestic), and whether wrapped in alfoil or Teflon.